

Turn on your tap and get hot- and cold-running crud.

Tom Lehrer, songwriter/satirist, "Pollution"

ECOLOGY AND WILDLIFE

Pharmed Fish

Results from a recent pilot study of the uptake of pharmaceuticals and personal care products (PPCPs) by fish in U.S. rivers have garnered considerable attention since they were published online 25 March 2009 ahead of print in *Environmental Toxicology and Chemistry*. The study, led by Baylor University chemistry professor C. Kevin Chambliss and environmental science professor Bryan Brooks, broke new ground by assessing chemical contamination of wild fish at a variety of sampling sites, screening a relatively large and diverse array of PPCPs.

The work focused on five rivers in Chicago, Dallas, Orlando, Phoenix, and West Chester (Pennsylvania) that receive wastewater directly from treatment facilities. All five rivers are considered "effluent-dominated" because the high volume of wastewater they receive continuously exposes their aquatic inhabitants to effluent-derived contaminants. "Such [worst-case] exposure scenarios are not represented in standardized toxicity tests used to assess ecological risk of PPCPs to aquatic life," Chambliss and Brooks point out in their paper.

The researchers screened samples of fish fillet (muscle and skin) for 24 pharmaceuticals and 12 personal care products, while samples of fish liver were screened for pharmaceuticals only. The team found levels of 7 pharmaceuticals and 2 personal care products above method detection limits in the tissues analyzed (see table). This is the first report of the presence of the lipid regulator gemfibrozil in U.S. wild fish.

The team explained that they chose the study sites because point source discharges from wastewater treatment facilities are "the most significant entry route for human pharmaceuticals into the aquatic environment." However, recent work by Joakim Larsson, an assistant professor at Göteborg University, published in the April 2009 issue of *Regulatory Toxicology and Pharmacology* suggest pharmaceutical manufacturing plants in the developing world can release much higher quantities into the nearby environment. Larsson says the impact of wastewater from pharmaceutical manufacturers in the developed world

is not yet clear, but a 19 April 2009 Associated Press story reports that unpublished data from the U.S. Geological Survey (USGS) and the U.S. Environmental Protection Agency (EPA) show U.S. plants also may emit high concentrations of pharmaceuticals.

In 2005, Chambliss and Brooks were part of the first team to document substantial uptake of pharmaceuticals in fish, and experts agree their latest findings are consistent with the work conducted to date by a number of scientists. Moreover, the findings have inspired the EPA, which funded the research, to expand its investigation of PPCPs in fish under its National Rivers and Streams Assessment to obtain a more detailed national perspective, says Suzanne Rudzinski, deputy director of the EPA Office of Science and Technology. The agency is investigating whether the concentrations of pharmaceuticals present in fish are of concern for human health or for the fish themselves, she says.

The very low concentrations of pharmaceuticals in the fish flesh may translate into "good news for public health concerns," says Shane Snyder, a project manager at the Southern Nevada Water Authority's Applied Research and Development Center. "You'd have to eat tons of fish to come even close to the pharmaceutical levels we predict would be of concern to human health," he says. He also points out that effluent-dominated waterways constitute an "amazing minority" of U.S. streams.

Dana Kolpin, a project chief with the USGS Toxic Substances Hydrology Program, is more cautious, however. "What if these compounds bioaccumulate in tissue or have unintended consequences from the complex mixtures of contaminants we're exposed to?" he asks. Moreover, he says, "USGS research has shown that PPCPs are commonly present in streams across the United States, not just those that are truly effluent-dominated. While

these results in effluent-dominated streams may be the extreme case, this will not be the only setting where such PPCPs will be found in fish tissue."

Last year, Kolpin and colleagues reported in the 15 March 2008 issue of *Environmental Science & Technology* that earthworms can take up PPCPs from biosolids applied on land. Considered together, the research to date begs for further study into the potential impacts of such chemical mixtures on both aquatic and terrestrial organisms, he says. This is particularly true because researchers' analytical methods allow them to look for only a few hundred of the thousands of PPCPs that might be in U.S. water bodies, adds Herb Buxton, coordinator for the USGS Toxic Substances Hydrology Program.

Comparing personal care products and pharmaceuticals, Snyder says the former may merit greater investigation—some of the concentrations documented by Chambliss and Brooks for these compounds are orders of magnitude higher than those seen for pharmaceuticals. For example, the highest uptake reported in the study was of the synthetic fragrance galaxolide, which showed a maximum concentration of 2,100 ng/g in fish fillets (sertraline,

Compound	Use	≥ MDL in Liver	≥ MDL in Fillet
Fluoxetine	Antidepressant	yes	no
Norfluoxetine	Antidepressant	yes	yes
Sertraline	Antidepressant	yes	yes
Diphenhydramine	Antihistamine	yes	yes
Diltiazem	Antihypertensive	yes	yes
Atenolol	Antihypertensive	no	no
Metoprolol	Antihypertensive	no	no
Propanolol	Antihypertensive	no	no
Carbamazepine	Antiseizure	yes	yes
Triclosan	Antimicrobial	no	no
Galaxolide	Fragrance	no	yes
Tonalide	Fragrance	no	yes
Celestolide	Fragrance	no	no
Gemfibrozil	Lipid regulator	yes	no
1,7 Dimethylxanthine	Antispasmodic	no	no
Acetaminophen	Analgesic	no	no
Ibuprofen	Analgesic	no	no
Caffeine	Stimulant	no	no
Cimetidine	Anti-acid reflux	no	no
Codeine	Analgesic	no	no
Erythromycin	Antibiotic	no	no
Lincomycin	Antibiotic	no	no
Sulfamethoxazole	Antibiotic	no	no
Trimethoprim	Antibiotic	no	no
Tylosin	Antibiotic	no	no
Miconazole	Antifungal	no	no
Thiabendazole	Antifungal	no	no
Warfarin	Blood thinner	no	no
4-Methylbenzylidene camphor	Ultraviolet filter	no	no
Octocrylene	Ultraviolet filter	no	no
m-Toluamide	Insect repellent	no	no
Musk ketone	Fragrance	no	no
Musk xylene	Fragrance	no	no
Nonylphenol	Surfactant	no	no
Octylphenol	Surfactant	no	no

the pharmaceutical consistently found at the highest concentrations, was detected in fillet and liver at 19 and 545 ng/g, respectively). The research literature makes it “pretty clear that [personal care products] are bioaccumulating,” Snyder says, adding that people often spray fragrance compounds all over themselves.

Larsson points out that although drugs are specifically designed to interfere with biological systems, synthetic musk fragrances are not. “Thus, even if levels are higher of the musks, it is not clearcut that the risks are higher with this group of chemicals,” he says.

Potential concerns raised by the new study include possible impacts on aquatic life in light of growing evidence that very low-level exposure to pharmaceuticals can impact fish and other underwater dwellers, Brooks and Kolpin point out. In time, research on the effects of PPCP exposure may inspire scientists to come up with new ways to conduct ecologic risk assessments, Brooks predicts. “Risk assessments are based on traditional responses in fish, such as changes in growth or survival, that we may not see when they’re exposed to PPCPs,” points out Karen

Kidd, a biologist with the University of New Brunswick’s Canadian Rivers Institute. For example, some PPCPs affect immune system function or behavior, responses that risk assessments don’t currently include, she says.

“The current testing approaches are not sufficiently optimized for efficiently finding either the species, the endpoints, or the drugs that are probably of most concern,” Larsson adds. He and Kidd agree that the new pilot study’s effort to document which compounds are taken up by exposed fish is an important step in the right direction. —Kellyn S. Betts

ENDOCRINE DISRUPTORS

Estrogens in a Bottle?

Much of our exposure to endocrine disruptors occurs through what we eat and drink—in some cases, chemicals such as plasticizers may have migrated from food or beverage packaging. The possibility that these chemicals end up in commonly consumed beverages was the focus of two recent European studies that found evidence of estrogenic activity in mineral water. Both studies focused on the estrogenic potential of mineral water bottled in polyethylene terephthalate (PET) plastic, the material constituting most convenience-size beverage bottles sold in the United States today.

In the first study, published in the March 2009 *International Journal of Hygiene and Environmental Health*, a recombinant yeast-based *in vitro* assay was used to assess estrogenic activity in 30 PET-bottled mineral water samples. Ninety percent of the samples tested negative for estrogenic activity. Of the remaining samples, most showed measurements corresponding to a range of 14–23 ng/L estradiol equivalents—similar to the estrogen burden posed by treated drinking water derived from groundwater and river water (15 and 17 ng/L estradiol equivalents, respectively).

Of the estrogen-positive samples, authors Barbara Pinto and Daniela Reali, investigators in the University of Pisa Department of Experimental Pathology, say the water may have been contaminated at its source, during processing, or after bottling. They cite several studies showing that suboptimal storage conditions—such as prolonged exposure to sunlight and high temperatures—can cause leaching of chemicals from PET bottles into fluid contents, and point out that “cell toxicity was



PET-housed snails produced up to twice as many embryos as glass-housed snails.

observed for water samples of the same lot of three different brands purchased from the same retailer.”

Estrogenic activity in PET-bottled mineral water was also observed by graduate student Martin Wagner and chairman Jörg Oehlmann of the Department of Aquatic Ecotoxicology at Johann Wolfgang Goethe University. Using a similar but more sensitive yeast-based estrogen screen, the researchers tested 20 brands of mineral water packaged

in PET, glass, or coated paperboard. Elevated estrogenic activity was measured in 12 of 20 brands of mineral water, including 78% of those bottled in PET and 33% of those bottled in glass. However, multiuse PET bottles (which are intended to be reused several times) showed lower estrogenicity than bottles meant for one-time use—and were even lower than glass bottles from the same mineral water source.

This study, published online 10 March 2009 in *Environmental Science and Pollution Research*, also included experiments in which mud snails (*Potamopyrgus antipodarum*), an organism that is highly sensitive to estrogens, were raised in glass and PET bottles. The findings mirrored those from the yeast-based assay, but with one interesting exception: A PET sample that showed minimal response in the yeast assay induced one of the more significant results in the mud snail assay.

The disparity implies bottled water may contain a mix of compounds. “Perhaps the snails were reacting to, for example, anti-androgens coming from these plastic bottles. We would have missed these *in vitro* because we only looked for [estrogen receptor] ligands,” Wagner says. Although he and Oehlmann also noted several points at which contamination could have occurred during water processing, Wagner says the

snail data led them to conclude that at least some contamination arose from the PET bottles: “Because the snail experiment did not use mineral water but rather a defined culture medium for snails, which was the same in all bottles, the estrogenic effect in the snails could only have come from the packaging material.”

This conclusion has been strongly discounted by several industry groups, including the PET Resin Association (PETRA). “It has been demonstrated through extensive studies that PET meets all established safety standards for use in food and beverage packaging and has been safely used for that purpose for decades,” says Ralph Vasami, executive director of PETRA. The organization also emphasizes that PET destined for food and beverage containers does not contain bisphenol A or orthophthalates, both of which have been heavily scrutinized as endocrine disruptors.

Still, we should think about the components of PET plastic in terms of potential leaching of products that have biological activity, says Kris Thayer, a staff scientist at the National Toxicology Program’s Center for the Evaluation of Risks to Human Reproduction, in response to the Italian and German studies. “If people are moving away from polycarbonate plastics [due to bisphenol A concerns], what do they use instead? When we consider alternative plastics, we need to be sure they are characterized,” she says. Part of the characterization process entails finding out which compounds, if any, leach from the plastic.

Neither of the European studies can be used to deduce anything about potential human health effects of drinking PET-bottled beverages. However, if PET bottles do leach endocrine-disrupting chemicals into the beverages they contain, it could represent a significant source of exposure for many people. According to figures from the Beverage Marketing Corporation published in the April/May 2009 issue of *Bottled Water Reporter*, Americans drank 108 L of bottled water per person in 2007, while per-capita Italian consumption reached 204 L. —Julia R. Barrett

HARMFUL ALGAL BLOOMS

An Unexpected Deep-Sea Diver

Domoic acid (DA), a potent neurotoxin produced primarily by the diatom genus *Pseudo-nitzschia*, is generated during harmful algal blooms—rapid surges in toxic algae populations that result from increases in nutrient availability, temperature, and sunlight, among other environmental changes. Previously, scientists assumed that once the blooms dissipated, DA was released into and diluted within the upper ocean layer. But a study published in the April 2009 issue of *Nature Geoscience* shows that DA can be trapped inside the silica shells of *Pseudo-nitzschia* and carried to the ocean floor. Concentrations of DA in particles collected in deep oceans were several times higher than the regulatory limit set by the U.S. Environmental Protection Agency (EPA) to prevent human poisoning.

DA can cause a condition called amnesic shellfish poisoning in people who eat contaminated crabs, oysters, clams, mussels, scallops, anchovies, and sardines—all of which feed on *Pseudo-nitzschia*. Symptoms include gastrointestinal upset, headache, dizziness, cardiac arrhythmia, coma, potential loss of short-term memory, and possibly death. Water-soluble DA concentrates in the stomachs of shellfish and tiny fish. To ingest DA, people must eat the whole organism, including the stomach, says Stephen Bates, phytoplankton scientist emeritus with Fisheries and Oceans Canada.

Although elderly people are considered the most vulnerable to the effects of DA, early-life exposures also may be problematic. Recent rodent studies by biologists at the University of Prince Edward Island, published in the 23 March and 16 April 2009 issues of *Physiology & Behavior*, found that neonatal exposure to low doses of DA was associated with lasting cognitive deficits and behavioral problems in adult animals. Moreover, findings reported in the December 2008 issue of *Marine Drugs* suggest DA may be immunotoxic in mice.

For the current study, researchers set up sediment traps off the coast of Southern California, where *Pseudo-nitzschia* blooms and DA poisoning are prevalent. The traps floated above the sea floor at depths of 540, 550, and 800 m. Sediment collected in traps set at 550 m contained up to 50 µg DA/g dry sediment, and traps set at 800 m contained up to 163 µg DA/g of dry sediment. Measurements showed that DA sank rapidly, settling to 800 m in about three days. Bates says shellfish and sediment levels are not directly comparable because the former reflects wet weight, whereas the latter reflects dry weight, but that a rough comparison can be made for the purpose of assessing relative amounts.

The findings suggest that marine creatures living in deeper waters may be contaminated with DA, yet health officials currently monitor only shellfish that live close to the surface. The U.S. EPA and the Canadian Food Inspection Agency regularly check commercial shellfish beds, with increased testing during algal blooms, closing the beds when levels reach 20 µg DA/g tissue. However, says study leader Claudia Benitez-Nelson, a geochemist at the University of South Carolina, “We no longer can use algal blooms as an indicator of [potential] DA poisoning.”

The fact that DA sinks to deeper waters may, in fact, help explain past mysterious outbreaks of shellfish poisoning. For instance, in 1995 lucrative deep-sea scallop beds were closed to harvesting off the coast of Nova Scotia in the Gulf of Maine. The scallops contained up to 3,400 µg DA/g tissue. “We didn’t know where the DA came from,” Bates says. “But the new data suggest that the cause could have been DA sinking down from surface blooms of *Pseudo-nitzschia*.”

The key to curbing DA poisoning is to understand why and when *Pseudo-nitzschia* blooms occur. Although harmful algal blooms are mainly viewed as natural phenomena, the magnitude and occurrence of some toxic species can be exacerbated by nutrient inputs from human sewage and fertilizer and possibly other forms of coastal pollution. “People are working hard to reduce runoff from crops and lawns, but it takes time,” says Benitez-Nelson. Meanwhile, she adds, “Once [*Pseudo-nitzschia*] bloom, it’s very difficult to control the toxins they produce.” —Carol Potera

The Beat by Erin E. Dooley

More Weight, More Allergies?

A study co-funded by the NIEHS and the NIAID suggests a possible connection between rising rates of childhood obesity and allergies over the past few decades. Analyzing 2005–2006 NHANES data, the researchers reported in the May 2009 *Journal of Allergy and Clinical*

Immunology that obese children were 26% more likely than children of normal weight to have allergies and 59% more likely to have a food allergy. Although an increased risk of allergy might not be the most serious health problem faced by overweight children, it does provide an added incentive for stepping up efforts to prevent childhood obesity, the researchers contend.

Some “Lead-Free” Pots Are Not

Traditional lead-glazed pottery from Mexico has been cited as a common source of lead exposure among people who use these wares. A study by the Environmental Quality Institute at the University of North Carolina at Asheville showed that Mexican-made “Mi Pueblo” brand clay pots marketed in some eastern U.S. states as “lead-free” actually contained nearly twice the amount of lead considered safe by the FDA for cups, mugs, and pitchers, and just under the safe level for serving bowls. Ironically, the researchers had hoped to confirm the safety of Mi Pueblo products to enable their promotion as a safe alternative to lead-glazed pots.



Military officers clean up the Qingdao algal bloom, July 2008

Massive Bloom the New Norm?

In summer 2008 an unprecedented algal bloom almost caused the cancellation of the Olympic Games’ sailing events in the regatta city of Qingdao, China. It took more than 10,000 people to clean up over 1 million tons of *Enteromorpha prolifera* algae from the beach and coast. Initially attributed to eutrophication, a study in the June 2009 *Marine Pollution Bulletin* now suggests a different provenance

Left to right: Anita Patterson/Stockphoto; Ng Han Guan/AP Photo

CHILDREN'S HEALTH

Gene Variants May Predict Lung Health

Genetic research is beginning to provide potential insights on why some people are more vulnerable than others to various pollutants. Now a few more pieces can be added to the extraordinarily complex puzzle. In the April 2009 *American Journal of Respiratory and Critical Care Medicine*, researchers from the University of Southern California report that several inherited glutathione S-transferase (GST) gene variants were associated with lung function growth during adolescence, and one variant helped predict poor lung function in children whose mothers had smoked during pregnancy.

GST genes are expressed in the lungs, among other organs, and may influence the development of normal lung function. This study focused on a class of GST genes called *GSTMs*. The best studied of these is *GSTM1*, which has been implicated in lung cancer, asthma, and other respiratory diseases in children and adults. To find out more about other variants, the authors looked at *GSTM2*, *GSTM3*, *GSTM4*, and *GSTM5*. They analyzed the genotypes in conjunction with eight years' worth of lung function and genotyping data from 2,108 Southern California schoolchildren, starting around age 10 years.

For *GSTM2*, two common haplotypes (patterns of genetic variation) were observed. One haplotype found in 28% of non-Hispanic whites and 44% of Hispanic whites was associated with better lung development than other haplotypes, whereas a second haplotype found in 35% of non-Hispanic whites and 30% of Hispanic whites was associated with significantly less lung function growth over time. Deficits in measures of lung function growth were twice as big in children with two copies of the haplotype versus those with one copy, and the deficits associated with this haplotype were greater among children of mothers who smoked during pregnancy.

The presence of a *GSTM4* haplotype found in 22% of non-Hispanic whites and 16% of Hispanic whites was associated with significantly reduced lung function growth relative to children with other

haplotypes. As with the *GSTM2* variant above, deficits in lung function growth were doubled among children with 2 copies of the haplotype. A *GSTM3* haplotype found in about 7% of the children was associated with a deficit in one of three measures of lung function growth.

The authors believe this to be the first study to report evidence of an interaction between *GSTM2* variants and prenatal tobacco smoke exposure on lung function growth. They noted that *GSTM* genes are expressed in fetal tissue at relatively low levels, and GSTs and other phase II (detoxification) enzymes are relatively inactive in fetal tissue compared with phase I enzymes (which activate toxic metabolites and carcinogens). "This suggests that fetuses are much more susceptible to environmental exposures than adults," they wrote. "Genetic polymorphisms in phase II enzymes that further inhibit enzyme activity may exacerbate this susceptibility." In addition, respiratory health and development in childhood is known to play an important role in adult respiratory health.

This study "points out again that the genetic makeup of a population may be very important when considering health risks from environmental exposures," says principal investigator Frank Gilliland, who directs the NIEHS-supported Southern California Environmental Health Sciences Center at the University of Southern California. His team speculates that *GSTM*-influenced failure to detoxify reactive oxygen species reduces lung protection and triggers an inflammatory cascade, bronchial constriction, and airway hyperresponsiveness, producing asthma-like symptoms and impaired lung development.

However, more information would be needed before public health officials could set or adjust regulatory standards based on variation in vulnerability among subpopulations. And even with such additional data, Medea Imboden, a molecular epidemiologist at the University of Zürich, cautions against overemphasizing the GST gene family. "You always have doubts about the functional relevance of the variants," says Imboden. "If you think of all the genes not yet known to contribute to respiratory health and bodily defense systems against air pollutants, GSTs might turn out to play a minor role." But for now, she adds, "they are good candidates." —Bob Weinhold

for the bloom. The authors used satellite images to trace the bloom's origin to the coast of Jiangsu province, where *E. prolifer* contaminates aquaculture operations that grow *Porphyra* seaweed for food. With the aquaculture industry off Jiangsu's coast having more than doubled in size since 2003, the authors say the stage is set for more massive blooms such as the one seen in Qingdao.

Carbofuran Banned for Food Crops

On 11 May 2009 the EPA revoked regulations that allowed small residues of the pesticide carbofuran on food crops, saying the compound poses an unacceptable health risk, especially to children and farmworkers. The move came after years of review, a partial ban in the mid-1990s (when millions of migratory bird deaths were linked to carbofuran), and an attempt earlier in 2009 by the chemical's manufacturers to delay further restrictions. The EPA cited unacceptable neurotoxicity and other health risks posed by ingesting residues of the insecticide in food and water. The final carbofuran tolerance rule takes effect in December 2009.

BPA Becoming Chemical *Non Grata*

In May 2009 Chicago and Minnesota became the first city and state, respectively, to ban bisphenol A (BPA) in baby bottles and children's cups. Lawmakers in Connecticut and California are now considering similar BPA regulations. On the federal level, bills aimed at banning BPA from all food and beverage containers were introduced in the House and the Senate in March 2009 but

are still in committee. BPA is found in some polycarbonate plastic containers such as water and baby bottles and in some food can linings. BPA is weakly estrogenic, and numerous animal studies suggest exposure to the compound during critical developmental windows may contribute to adverse reproductive, behavioral, and metabolic effects.

Bad Air Rising

Six of 10 U.S. citizens, or more than 186 million people, reside in areas with dangerous levels of air pollution, according to the American Lung Association's *State of the Air: 2009* report. Although some major U.S. cities have improved their air quality over the last decade, the air in many other cities became more polluted last year. The 2009 figures are substantially higher than those from 2008, when an estimated 42% of the population faced similar conditions. The report includes a call for the EPA to strengthen its standards for ozone and particulate matter. The agency issued new ozone standards in 2008, and proposed revisions to particulate matter standards are due in 2010.

